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ROOT DECAY OF CONTAINER-GROWN WESTERN WHITE PINE SEEDLINGS PLUM CREEK NURSERY, PABLO, MONTANA

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ABSTRACT

Root decay of container-grown western white pine seedlings at the Plum Creek Nursery in Pablo, Montana was common in the 1987 and 1988 crops, lessening in 1989. Affected seedlings lacked above-ground disease symptoms, although they were usually smaller than seedlings without decay. Several sets of isolations indicated that *Cylindrocarpon* spp. (mostly *C. didymum* and *C. tenue*) were consistently associated with decayed roots. These fungi were also isolated from healthy-appearing roots and the inner walls of styroblock containers. *Fusarium* spp. were isolated much less frequently. It was concluded that *Cylindrocarpon* spp. were most likely the cause of root decay, although controlled pathogenicity tests are required to confirm this conclusion. Approaches to reducing future losses are discussed.

INTRODUCTION

Western white pine (*Pinus monticola* Dougl. ex D. Don) is an important reforestation conifer species in the northern Rocky Mountains. The Plum Creek Nursery at Pablo, Montana annually produces about 200,000-300,000 container-grown white pine seedlings for outplanting on the lands. Genetically improved white pine seed is collected from seed orchards comprised of parents showing resistance to blister rust.

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In the fall of 1987 when white pine seedlings were extracted from containers for over-winter storage, 5-10 percent of the stock was unsuitable for shipment because of extensively decayed root systems, even though above-ground disease symptoms were absent. Some affected seedlings had very few roots, even though foliage was green and seedlings appeared normal before extracting (Figure 1 - Appendix). Most affected seedlings had many normal-appearing roots, but portions of root systems were decayed, especially near the bottom of plugs (Figure 2 - Appendix). Decayed roots often had an intact stele; cortical and epidermal cells had been decayed and sloughed off.

The Plum Creek Nursery is relatively new and lacks a history of disease problems. For the 1987 crop, preventative fungicide applications were made to reduce damage from damping-off and early root disease which usually occurs at low levels. Fungicides used included metalaxyl, benomyl, captan, and Banrot®. Daconil was applied in November for *Botrytis* control after seedlings had hardened. However, no *Botrytis* was found and the crop looked very good prior to lifting.

Similar root damage was also noticed in the 1988 and 1989 white pine crops, but damage levels were generally less than those found in 1987. Losses were estimated at about 5 percent for the 1988 crop and less than 1 percent in 1989.

Growers were concerned about these losses because white pine is a preferred reforestation species and high-quality stock is needed. They wanted an evaluation of the possible cause(s) of the root decay and alternatives available for reducing future damage. This report summarizes findings on organisms associated with root decay and those isolated from seed and containers that might be involved in the problem.

MATERIALS AND METHODS

Seedlings with various levels of root decay were sampled from the 1987, 1988, and 1989 crops. Each analyzed seedling was rated for extent of root decay as either severe, moderate, or slight (see Table 1 in the Appendix for decriptions of ratings). Five isolation sets were conducted over 3 years (see Table 2 in the Appendix for descriptions). All roots selected for isolation had some evidence of decay. Decay was often concentrated on root tips (Figure 2 - Appendix). Roots with decay (stele remaining without cortex) were aseptically severed and pieces 2-3 mm long were cut from just above the exposed stele (where active decay would be expected). Normally, one root piece was sampled from each decayed root; 4-15 roots were sampled per seedling (Table 2 - Appendix). Root pieces were surface sterilized in a 10 percent bleach solution (0.525% aqueous sodium hypochlorite) for 1 minute and rinsed in sterile distilled water. They were then placed on a selective agar medium for *Fusarium* and related fungi (Komada 1975), as well as another selective medium for water molds such as *Pythium*, *Phytophthora*, and related fungi (V-8 juice agar amended with pimaricin). Plates with Komada's medium were incubated for 7-10 days at about 26°C under diurnal cycles of cool, fluorescent light. Plates with V-8 juice agar were incubated at about 24°C in the dark for 3 days. Fungi emerging from root pieces were identified after transfer to either potato dextrose, carnation leaf, or cornmeal agar amended with antibiotics for identification. *Fusarium* spp. were identified using the taxonomic systems of Nelson and others (1983) and Gerlach and Nirenberg (1982); *Cylindrocarpon* spp. were classified based on decriptions by Booth (1966). Other fungi were identified using different taxonomic descriptions (Barnett and Hunter 1972; Domsch and others 1980; Dorenbosch 1970).

Isolation set 5 (Table 2 - Appendix) involved growing seedlings with moderate or slight amounts of root decay within pots on greenhouse benches. Ten seedlings (five each with moderate and slight root decay) were transplanted into pots using standard peat-vermiculite growing media (Forestry Mix® - W. R. Grace & Co.) in April 1989. Seedlings were watered as needed and monitored for foliar symptoms indicative of root disease. Two seedlings died in June (one each with moderate and slight root decay). The others grew throughout the summer without developing above-ground disease symptoms. Dead seedlings were analyzed for root infection by potentially pathogenic fungi shortly after death; surviving seedlings were analyzed at the end of the growing season (November). Roots of all seedlings were washed, small pieces extracted, surface sterilized, and incubated on Komada's medium as described above. Twenty-five root pieces were sampled per seedling. Emerging fungi were identified as described above.

For seedlings analyzed in isolation set 3 (Table 2 - Appendix), two additional isolation types were attempted. Root tips from non-decayed roots (those without exposed stele) were severed, surface sterilized, and incubated on Komada's agar as described above. Within the same root systems, exposed stele tissue was likewise sampled. Emerging fungi were identified as described above.

Five seedlings from the 1987 crop without any evidence of root decay were analyzed for fungal colonization of roots. Fifteen randomly-selected root tips were aseptically sampled per seedling as described previously. Emerging fungi were identified.

Seedlings from isolation set 1 (Table 2 - Appendix), including the five without root decay, were measured (height from groundline to tip of terminal bud and caliper just above the groundline) and above-ground oven-dry weights determined to evaluate possible relationships between root decay severity and seedling growth.

Container-grown seedlings at the Plum Creek Nursery are grown in styroblock containers. Such containers are substrates for root disease fungi, and when reused, infect new crops of seedlings (James and others 1988a; Sturrock and Dennis 1988). Therefore, eight randomly selected styroblock containers from the 1988 crop were sampled. Four blocks were "cleaned" by immersion for 30 seconds in a 2.5 percent aqueous solution of sodium metabisulfite (which had proved efficaceous in previous tests by Sturrock and Dennis (1988)) and allowed to air dry. The other four blocks remained untreated. Ten cells per block were randomly sampled. At the bottom of each cell, four styrofoam pieces (2-3mm^2) were aseptically extracted (one from each of the four cardinal directions) and incubated on Komada's medium as described above. Emerging fungi were identified as described above.

Another possible inoculum source was contaminated seed (James 1986). Therefore, seed from the 1988 cone crop produced at the Moscow Arboretum were sampled for fungal contamination. Fifty processed (Syverson Seed, Inc., Ridgefield, WA) seed were aseptically incubated on Komada's medium and emerging fungi identified.

RESULTS AND DISCUSSION

Isolation results are summarized in the Appendix. Isolations onto Komada's medium from most decayed roots in all five sets of seedlings yielded high percentages of *Cylindrocarpon* spp. (Table 3). Colonization intensity of *Cylindrocarpon* spp. was generally higher in more severely decayed root systems. Although *Fusarium* spp. may be common root pathogens of container-grown white pine seedlings (James 1985b, 1987a), relatively low levels of these fungi were isolated from decayed roots. Other groups of fungi isolated from decayed roots included common saprophytes (*Trichoderma*, *Penicillium*, *Alternaria*) and *Phoma* spp., which may sometimes be parasitic on conifer seedlings (James 1985a; James and Hamm 1985). Isolations from decayed roots on V-8 juice agar

amended with pimaricin (selective for water mold-type fungi), yielded fairly high levels of *Mortierella* spp. (Table 4), but the common water mold pathogens of conifer seedlings, *Pythium* and *Phytophthora* spp. (Sutherland and others 1989), were absent.

Isolations from non-decayed root tips and root stele tissues (Tables 5 and 6, respectively), also yielded fairly high levels of *Cylindrocarpon* spp. *Fusarium* levels were again generally low, although common saprophytes (*Trichoderma* and *Penicillium*) were well represented. *Cylindrocarpon* spp. were also often isolated from seedling roots without noticeable root decay (Table 7). *Fusarium* spp. were also found on most of these seedlings, but at low intensities.

Seedlings with severe root decay were usually shorter and had less above-ground biomass than those with less decay (Table 8), although small sample sizes inhibited statistical comparisons. These differences probably indicate growth affects, i.e., seedlings with root decay were not growing as well as those without decay.

Most seedlings with moderate or slight amounts of root decay survived transplanting (under greenhouse conditions) for at least one growing season. At the end of this test, root decay was still evident and *Cylindrocarpon* spp. still found at high levels on seedling roots (Table 3, isolation set 5).

Cylindrocarpon spp. were detected at very high levels on styroblock containers used during the 1988 crop (Table 9). Treating containers with sodium metabisulfite was ineffective in reducing amounts of *Cylindrocarpon* and other fungi. Because containers had high fungal contamination, growers used new styroblocks for the 1989 crop of white pine. Although the level of root decay was less in this crop, some decay was still found. Levels of seedborne *Cylindrocarpon* (Table 10) were quite low; other possible inoculum sources were not investigated. Peat-vermiculite growing media is usually devoid of pathogens (James 1985c). Infested soil from nearby fields might provide some inoculum; grass, weeds and other nearby plants may also harbor inoculum (Booth 1966; Matturi and Stenton 1964; Ross 1960). Since greenhouse walls at the Plum Creek Nursery are often rolled up during much of the growing season, surrounding air movements may bring root decay inoculum into greenhouses. However, root decay has only consistently been encountered in western white pine stock. Introduced inoculum levels may be low or white pine seedlings may be especially susceptible to the organisms causing decay.

Cylindrocarpon didymum (Hartig) Wollenw., *C. tenue* Bugn., and *C. gracile* Bugn. were isolated from white pine roots and seeds and styroblock containers. The first two species were previously implicated in conifer seedling diseases (Booth 1966; Houten 1939; James 1988a; James and Gilligan 1985), but *Cylindrocarpon* spp. are usually not considered aggressive pathogens like some *Fusarium* spp. (Booth 1966; James 1988a). Although observed for several consecutive crop cycles as consistently associated with decayed root systems of white pine seedlings, the ability of *Cylindrocarpon* spp. to initiate disease symptoms in conifer seedlings needs to be determined.

The most common *Fusarium* species isolated from roots, seeds, and containers was *F. oxysporum* Schlecht. This is a common pathogen of container-grown conifer seedlings, including western white pine (James 1985b, 1987a, 1987b, 1988b). Other fusaria isolated included *F. acuminatum* Ell & Ev., a common root pathogen (James and others 1989), *F. sambucinum* Fuckel, most likely a saprophyte (James and others 1989) and two other species (*F. culmorum* (W. G. Smith) Sacc. and *F. chlamydosporum* Wollenw. & Reinking) not usually found on conifer seedlings (Nelson and others 1983). Although many *Fusarium* spp. can cause diseases of conifer seedlings (James 1986; James and others 1989), several also occur on non-diseased seedlings (James and Gilligan 1988a, 1988c), possibly as saprophytes. *Fusarium* spp. were found at relatively low levels on container-grown white pine seedlings at the Plum Creek Nursery and were probably not the major cause of root decay.

Other fungi isolated in this investigation were also probably saprophytic. *Trichoderma* spp. are very common colonizers of cortical root tissues, seedcoats, and the interior of styroblock containers (James and others 1987; James and Gilligan 1988b). Although common saprophytes, *Trichoderma* spp. may be antagonists or competitors of pathogenic fungi (Papavizas 1985). *Penicillium* and *Alternaria* spp. are common saprophytic rhizosphere colonizers (Domsch and others 1980). *Phoma* spp. may be saprophytic or pathogenic, depending on environmental, host, and pathogen characteristics (James and Hamm 1985). The two species encountered in this investigation were *P. herbarum* Westend. and *P. eupyrena* Sacc. Both have been associated with conifer seedling diseases, although *P. eupyrena* is more often implicated as a pathogen (James 1985a; James and Hamm 1985).

Mortierella spp. were isolated on V-8 juice agar at relatively high levels from seedlings with different amounts of root decay in isolation sets 1 and 2 (Table 4). These fungi are common soil fungi (Domsch and others 1980), usually decomposing of soil organic matter (Jackson 1965). Although frequently isolated from the rhizosphere of conifer species (Hendrix and others 1971; Lihnell 1939; Svinnufrud 1937), *Mortierella* spp. are not implicated as causes of plant disease (Domsch and others 1980).

To summarize, this investigation has shown that *Cylindrocarpon* spp. were consistently associated with root decay symptoms of container-grown western white pine seedlings at the Plum Creek Nursery. Although other fungi were also encountered, they were found less frequently and many are without a history of pathogenic behavior on conifer seedlings (except *Fusarium* and *Phoma* spp.). Tests to evaluate potential of *Cylindrocarpon* spp. to cause root decay are needed to confirm their role in this disorder.

MANAGEMENT IMPLICATIONS

Because above-ground symptoms are usually absent with root decay, it is impossible for growers to predict losses before seedling extraction. Although it appears seedlings with low levels of root decay might survive outplanting, growers are reluctant to ship seedlings with noticeable root decay. Because fungicide treatments to control root disease in container operations have largely been unsuccessful (James and others 1988b), the best means of reducing future losses from *Cylindrocarpon* spp. is prevention of infection.

In order to successfully prevent infection, inoculum sources and conditions conducive for seedling infection should be known. Although our knowledge of the epidemiology of this disease is limited, growers can reduce infection. Fortunately, seed and growing medium seem insignificant sources of inoculum. Reused, inadequately cleaned containers will probably harbor significant levels of inoculum. Growers should continue to use new or adequately-cleaned containers for white pine and conduct preventative fungicide treatments for damping-off and root disease as in the past. Roguing of dead seedlings may also be important in minimizing inoculum build-up.

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APPENDIX

Figure 1. Container-grown western white pine seedling with extensive root decay - Plum Creek Nursery. Note that seedling foliage appears normal.

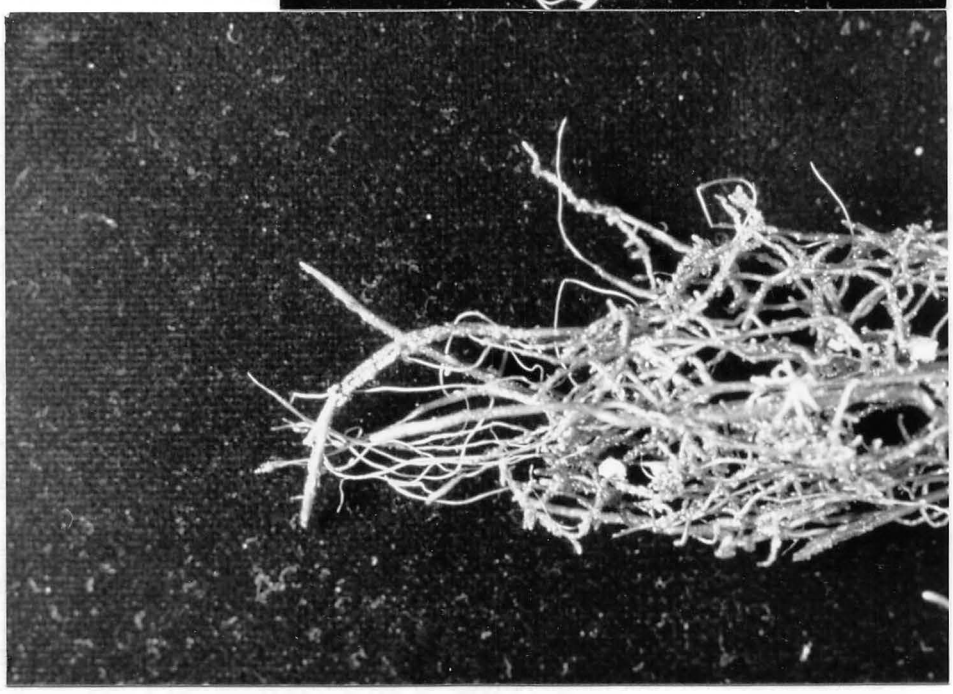

Figure 2. Decayed roots of container-grown western white pine seedling - Plum Creek Nursery. Decayed roots have lost cortical and epidermal tissues whereas steles are largely in tact.

Table 1. Descriptions of root decay severity ratings for container-grown western white pine seedlings - Plum Creek Nursery

Root Decay Severity	Description
Severe	At least 33 percent of the root system with decay as evidenced by loss of cortical and epidermal tissues (exposed stele). Decay can occur throughout root system, but is usually concentrated either just below the soil line or at the bottom of the plug.
Moderate	From 10 percent to 33 percent of the root system with noticeable decay. Concentrated areas of decay are either just below the soil line or at the bottom of the plug.
Slight	Less than 10 percent of the root system with noticeable decay. Decayed portions of roots ususally concentrated near the bottom of plug.

Table 2. Description of isolation sets for container-grown western white pine seedlings with root decay - Plum Creek Nursery

Set No.	Description
1	Seedlings from 1987 crop; analysis conducted 12/87; 26 seedlings sampled; 15 sections of decayed root sampled per seedling.
2	Seedlings from 1988 crop; analysis conducted 12/88; 12 seedlings sampled; 6-10 sections of decayed root sampled per seedling.
3	Seedlings from 1989 crop; analysis conducted 10/89; 9 seedlings sampled; 4-10 sections of root sampled per seedling.
4	Seedlings from 1989 crop; analysis conducted 12/89; 9 seedlings sampled; 10 sections of decayed root sampled per seedling.
5	Seedlings from 1988 crop; 10 seedlings (5 each with moderate and slight root decay - Table 1) transplanted into pots 4/89, kept on greenhouse benches, watered as needed. Two seedlings (one each with moderate and slight root decay) died 6/89 and analyzed for root fungi; remaining seedlings analyzed 11/89; 25 sections of decayed root sampled per seedling.

Table 3. Fungi isolated from decayed roots of western white pine.

Root Decay Severity/ Isolation Set[1]	Number Seedlings Sampled	Percent Colonization[2] Isolated Organisms[3]									
		CYL		FUS		TRI		PEN		PHO	
		S	I	S	I	S	I	S	I	S	I
Severe											
1	11	100	98.8	100	9.7	27	7.2	0		0	
2	4	100	72.4	0		0		0		0	
3	2	100	42.9	0		50	7.1	50	7.1	50	7.1
4	2	100	95	0		100	20.0	0		0	
Subtotal	19	100	91.7	58	7.0	32	7.5	5	0.4	5	0.4
Moderate											
1	8	100	95.0	50	5.8	50	8.3	0		0	
2	4	100	70.6	0		25	5.9	25	5.9	0	
3	2	100	62.3	0		0		100	14.3	0	
4	2	100	100.0	50	5.0	100	10.0	0		0	
5	5	100	88.8	60	5.6	100	35.2	100	74.4	60	5.6
Subtotal	21	100	72.5	38	3.8	57	18.5	38	31.0	24	3.5
Slight											
1	7	100	84.8	43	4.8	0		0		0	
2	4	100	88.2	0	0		25	2.9	0		
3	5	60	14.8	0		20	11.1	20	3.7	60	14.8
4	5	40	4.0	20	2.0	100	84.0	40	4.0	40	4.0
Subtotal	26	81	64.5	27	5.6	42	23.7	35	23.5	27	2.9
Totals	66	92	80.0	39	5.3	44	17.7	27	20.2	20	2.5

[1] See table 1 for descriptions of root decay severity ratings and table 2 for descriptions of isolation sets.

[2] Isolations made from decayed roots with attached cortex onto Komada's medium. *S* = percent of sampled seedlings colonized with appropriate fungi; *I* = colonization intensity (percent of root pieces colonized).

[3] CYL = *Cylindrocarpon* spp., including *C. didymum, C. tenue* and *C. gracile*
FUS = *Fusarium* spp.; primarily *F. oxysporum* and *F. acuminatum*
TRI = *Trichoderma* spp.
PEN = *Penicillium* spp.
PHO = *Phoma* spp., including *P. eupyrena* and *P. herbarum*.

Table 4. Isolation of *Mortierella* spp. from decayed roots of container-grown western white pine seedlings - Plum Creek Nursery.

Root Decay Severity[1]	Isolation Set[2]	No. Seedlings Sampled	Percent Seedlings Colonized	Colonization Intensity
Severe	1	11	100	36.4
	2	4	100	78.4
	3	2	0	0
	4	2	0	0
	Subtotal	19	79	40.3
Moderate	1	8	25	5.2
	2	4	100	63.0
	3	2	0	0
	4	2	0	0
	Subtotal	16	38	22.7
Slight	1	7	57	10.7
	2	4	100	77.5
	3	5	0	0
	4	5	0	0
	Subtotal	21	38	30.3
	Totals	56	52	28.2

[1] See table 1 for descriptions of root decay severity.

[2] See table 2 for descriptiosn of isolation sets.

Table 5. Fungi isolated from non-decayed root tips of container-grown western white pine seedlings with various levels of root decay - Plum Creek Nursery.

	Percent Colonization[1] Root Decay Severity[2]							
Isolated Organism	Severe		Moderate		Slight		Totals	
Cylindrocarpon	100	45.0	100	50.0	80	26.0	89	35.5
Fusarium oxysporum	50	10.0	0	--	60	8.0	44	6.7
Trichoderma	100	20.0	50	5.0	80	10.0	78	11.1
Penicillium	0	--	0	--	60	8.0	38	4.4
Phoma	50	20.0	50	10.0	60	16.0	55	15.5

[1] Isolations made from the tips of non-decayed roots onto Komada's medium (decayed roots were within the root system of sampled seedlings). S = percent of sampled seedlings colonized with appropriate fungi; I = colonization intensity (percent of root pieces colonized).

[2] See table 1 for descriptions of root decay severity. Sample sizes: severe = 1 seedlings; moderate = 2 seedlings; slight = 5 seedlings; 10 root tips sampled per seedling.

Table 6. Fungi isolated from root steles of western white pine seedlings with various levels of root decay - Plum Creek Nursery.

Isolated Organism	Percent Colonization[1] Root Decay Severity[2]							
	Severe		Moderate		Slight		Totals	
	S	I	S	I	S	I	S	I
Cylindrocarpon	50	23.1	50	61.5	20	4.0	33	23.5
Fusarium oxysporum	0	--	50	15.4	20	4.0	22	5.9
Fusarium acuminatum	0	--	0	15.4	20	4.0	11	2.0
Trichoderma	0	--	50	7.7	40	12.0	33	7.8
Pinicillium	59	7.7	0	--	0	--	11	2.0

[1] Isolations made from root stele lacking cortex and epidermis (figure 2) onto Komada's medium within root system with decayed roots. S = percent of sampled seedlings colonized with appropriate fungi; I = colonization intensity (percent of root pieces colonized).

[2] See table 1 for descriptions of root decay severity. Sample sizes: severe = 2 seedlings; moderate = 2 seedlings; slight = 5 seedlings; 4-8 stele pieces sampled per seedling.

Table 7. Fungi isolated from root systems of container-grown western white pine seedlings without decay symptoms - Plum Creek Nursery.

	Percent Colonization[1] Isolated Organisms					
No. seedlings Sampled	*Cylindrocarpon*		*Fusarium*		*Trichoderma*	
	S	I	S	I	S	I
5	100	97.3	60	5.3	40	6.7

[1] Isolates made from randomly selected roots onto Komada's medium. S = percent of sampled seedlings colonized with appropriate fungi; I = colonization intensity (percent of root pieces colonized).

Table 8. Effects of root decay severity on height, caliper, and top dry weight of container-grown western white pine seedlings - Plum Creek Nursery.

	Height (mm)		Caliper (mm)		Top Dry Weight (g)	
Root Decay Severity[1]	Avg.	Range	Avg.	Range	Avg.	Range
Severe	103.4	68 - 171	3.54	3.0 - 4.0	1.027	0.532 - 2.315
Moderate	118.4	71 - 169	3.50	3.0 - 4.0	0.774	0.605 - 1.009
Slight	110.6	88 - 151	3.43	3.0 - 4.0	1.228	0.556 - 2.046
None	176.0	112 - 218	4.60	4.0 - 5.0	2.746	1.855 - 3.838

[1] See table 2 1 for descriptions of root decay severity. Sample sizes: severe = 11 seedlings; moderate = 8 seedlings; slight = 7 seedlings, none = 5 seedlings. Sample sizes insufficient for statistical comparisons.

Table 9. Fungal colonization of styroblock containers from the Plum Creek Nursery.

	Percentage Colonization[1]			
	Uncleaned		Cleaned[2]	
Fungus	C	I	C	I
Cylindrocarpon[3]	86.2	80.0	87.5	73.7
Fusarium[4]	3.7	1.9	1.9	2.5
Trichoderma	10.0	6.9	25.0	15.0
Phoma[5]	11.2	6.9	10.0	6.2
None Detected	11.2	6.9	12.5	7.5

[1] Eight randomly selected styroblock containers were sampled; 10 cells were randomly sampled from each container. C = percent of sampled cells colonized with appropriate fungi; I = colonization intensity (percent of styrofoam pieces colonized - 4 sampled per cell).

[2] Cleaning involved immersing styroblock containers in a 2.5 percent aqueous solution of sodium metabisulfite for 30 seconds.

[3] Combination of *C. didymum* and *C. tenue*.

[4] Combination of *F. oxysporum, F. acuminatum*, and *F. sambucinum*.

[5]Combination of *P. herbarum* and *P. eupryena*.

Table 10. Occurrence of selected fungi on western white pine seed - Plum Creek Nursery.

Fungus	Percent Colonization*
Fusarium oxysporum	6
Fusarium tricinctum	2
Fusarium chlamydosporum	2
Fusarium culmorum	2
All *Fusarium*	12
Cylindrocarpon gracile	2
Phoma herbarum	6
Botrytis cinerea	2
Trichoderma spp.	20
Alternaria spp.	4
Penicillium spp.	100

*50 seeds sampled; colonization of seedcoat.